FORMULATIONS FOR PARENTERAL USE OF ESTRAMUSTINE PHOSPHATE WITH IMPROVED PHARMACOLOGICAL PROPERTIES

The present invention relates to pharmaceutical formulations of estramustine phosphate for parenteral use with improved pharmacological properties and, more particularly, to formulations of estramustine phosphate for parenteral use further comprising sulfoalkyl ether cyclodextrins and human albumin.

Estramustine phosphate (The Merck Index, XII Ed., No. 3749, 1996) is an estradiol-17β-phosphate derivative widely known in the art as antitumor agent, currently used in the treatment of advanced adenocarcinoma of the prostate.
The drug is usually administered orally, preferably at a dose of 10-15 mg/kg/day. Intravenous administration, however, is also adopted in some particular cases.
For example, initial intravenous administration of estramustine phosphate, followed by oral administration, has been reported at dosages paralleling the oral administration for the drug, i.e. 300-600 mg daily given intravenously and usually repetitively over for several consecutive days (see, for a reference, British Journal of Urology, 1977, 49, 73-79; J. Urol.108:303-306, 1972; Eur. Clin. Pharmacol. 26(1), 113-119, 1984; Eur. Urol. 1990, 17, 216-218).

Estramustine phosphate as well as other well-known cytotoxic compounds used in antitumor therapy are known to cause, or potentially cause, vascular damages at the site of injection when parenterally, in particular intravenously, administered.
As an example, studies in patients treated with estramustine phosphate administered as a slow intravenous injection or as a bolus, at 300 mg/day, revealed

thrombophlebitis and local irritations at the peripheral intravenous injection sites.

These drawbacks are considered major limitations for the intravenous administration of estramustine phosphate, thus requiring, in many patients, the establishment of central line administration or, in some cases, even discontinuation of the treatment.

With the aim of minimising the unwanted effects associated with the intravenous administration of cytotoxic agents, a few means are reported in the art.

Among them is the use of cyclodextrins, for instance hydroxypropyl-cyclodextrin, in the preparation of formulations for parenteral administration of cytotoxic known to cause ulcerative lesions. See, for a reference, US patent No. 5,804,568 in the name of Supergen Inc.

Cyclodextrin derivatives such as sulfoalkyl ether cyclodextrins are known in the art as solubilizing agents for insoluble or poorly soluble drugs (see, for a reference, US 5,134,127 in the name of the University of Kansas).

Also known in the art are formulations for the intravenous administration of estramustine phosphate containing human albumin, reported to be characterised by fewer local side-effects upon injection of the active (see, for a reference, H. Schutz et al.; Krankenhauspharmazie, II year, issue No. 3, 1988).

In this respect, we found formulations for parenteral use comprising estramustine phosphate together with sulfoalkyl ether cyclodextrins and human albumin which, unexpectedly, resulted to achieve optimal protection from side-effects associated with estramustine administration.

It is therefore the object of the present invention a formulation for parenteral use comprising estramustine

phosphate in admixture with a sulfoalkyl ether cyclodextrin and human albumin.

Once administered intravenously to patients, the formulations object of the present invention do not provoke ulcerative damages, nor thrombophlebitis, at the site of injection.

Very interestingly, the estramustine phosphate formulations of the invention result to be endowed with unexpected pharmacological properties, expressed in terms of toxicity at the site of injection, markedly improved with respect to formulations containing, as a single protective excipient, a sulfoalkyl ether cyclodextrin or, alternatively, human albumin.

In the present invention, unless otherwise specified, with the term formulation comprising estramustine phosphate, as the active ingredient, we intend any formulation comprising estramustine phosphate either in the acid form or as a pharmaceutically acceptable salt for parenteral administration such as, for instance, a salt with a basic amino acid or with N-methyl glucamine, otherwise referred to as meglumine.

Preferably, estramustine phosphate is in the form of its meglumine salt.

With the term sulfoalkyl ether cyclodextrin we refer to any cyclodextrin of the above type wherein alkyl stands for straight or branched C_1-C_6 alkyl group such as methyl, ethyl, n.propyl, isopropyl, n.butyl, isobutyl, sec-butyl, tert-butyl, n.pentyl, n.hexyl and the like.

Preferably, the formulation of the present invention comprises estramustine phosphate in admixture with sulfobutyl ether ß-cyclodextrin.

According to a preferred embodiment of the invention, the weight ratio between estramustine phosphate and sulfoalkyl

ether cyclodextrin is comprised from about 1:0.5 to about 1:5, respectively.

However, higher amounts of sulfoalkyl ether cyclodextrin with respect to the active are sill effective and hence comprised within the scope of the present invention.

According to another preferred embodiment of the invention, the above formulations are advantageously used for intravenous use.

As such, these formulations of the invention can be administered to patients either as a slow injection, e.g. over about 30 minutes to about 3 hours, or as a bolus injection, also referred to as IV (intravenous) push.

In another preferred embodiment of the invention either

(i) the estramustine phosphate is in lyophilised form and the parenterally acceptable carrier or diluent is a physiological solution for parenteral use containing the sulfoalkyl ether cyclodextrin and the human albumin, or

(ii) the estramustine phosphate and sulfoalkyl ether cyclodextrin are in lyophilised form and the parenterally acceptable carrier or diluent is a physiological solution for parenteral use containing the human albumin.

The invention also provides a product which comprises estramustine phosphate in lyophilised form and a physiological solution for parenteral use containing human albumin.

The formulations of the invention also provide a very advantageous method for delivering estramustine phosphate intravenously, even when high doses of the active are needed.

It is therefore a further object of the invention a formulation for parenteral use comprising estramustine

phosphate, as a single infusion dosage of the active exceeding 1300 mg, in admixture with a sulfoalkyl ether cyclodextrin and human albumin.

According to another preferred embodiment of the invention, it is further provided a formulation for parenteral use comprising estramustine phosphate, as a single infusion dosage of the active exceeding 950 mg/m^2, in admixture with a sulfoalkyl ether cyclodextrin and human albumin.

The formulations object of the present invention allow the administration of the active either as a single agent or, alternatively, in combination with known anticancer treatments such as radiation therapy or chemotherapy regimen in combination with cytostatic or cytotoxic agents, antibiotic-type agents, alkylating agents, antimetabolite agents, hormonal agents, e.g. aromatase inhibitors, immunological agents, interferon-type agents, cyclooxygenase inhibitors (e.g. COX-2 inhibitors), metallomatrixprotease inhibitors, telomerase inhibitors, tyrosine kinase inhibitors, anti-growth factor receptor agents, anti-HER agents, anti-EGFR agents, anti-angiogenesis agents, farnesyl transferase inhibitors, ras-raf signal transduction pathway inhibitors, cell cycle inhibitors, other cdks inhibitors, tubulin binding agents, topoisomerase I inhibitors, topoisomerase II inhibitors, and the like.

As an example, the above formulations can be administered in combination with one or more chemotherapeutic agents, optionally within liposomal formulations thereof.

Examples of chemotherapeutic agents are, for instance, taxane, taxane derivatives, CPT-11, camptothecin and derivatives thereof, anthracycline glycosides, e.g. doxorubicin, idarubicin or epirubicin, etoposide, navelbine, vinblastine, carboplatin, cisplatin and the like, optionally within liposomal formulations thereof.

In addition, the above formulations can be also administered in combination with protein kinase inhibitors such as, for instance, the indolinone derivatives disclosed by Sugen in the international patent applications WO 96/40116 and WO 99/61422, which are herewith incorporated by reference.

In this respect, the formulations object of the invention can be preferably administered in combination with 3-[4-(2-carboxyethyl-3,5-dimethylpyrrol-2-yl)methylidenyl]-2-indolinone and 3[(2,4-dimethylpyrrol-5-yl)methylidenyl]-2-indolinone, better known as Sugen SU 6668 and SU 5416, respectively.

The formulations of the invention may be administered sequentially with known anticancer agents when a combination formulation is inappropriate.

Therefore, it is a further object of the present invention a product containing a formulation for parenteral use of estramustine phosphate in admixture with a sulfoalkyl ether cyclodextrin and human albumin and one or more chemotherapeutic agents, as a combined preparation for simultaneous, separate or sequential use in anticancer therapy.

Toxicology

To study the local irritant effects of estramustine phosphate after repeated intravenous administrations to rats, in comparison to a formulation of estramustine phosphate according to the present invention, the active was dissolved in different vehicles such as water solution for injection and water solution for injection further containing sulfobutyl ether ß-cyclodextrin and human albumin. In addition, formulations of estramustine phosphate in admixture with sulfobutyl ether ß-cyclodextrin only or, alternatively, with human albumin only, were used for comparison.

In particular, the following water for injection solutions wherein either the active estramustine phosphate (therein referred to as EMP) as well as the excipients sulfobutyl ether ß-cyclodextrin and human albumin (therein referred to as SBECD and HA, respectively) are expressed in terms of weight ratios, were prepared and tested:

(a)	**negative control:**	**water for injection;**
(b)	**positive control:**	**EMP;**
(c)	**comparison:**	**EMP:SBECD=1:1;**
(d)	**comparison:**	**EMP:HA=1:0.21;**
(e)	**tested solution:**	**EMP:SBECD:HA=1:1:0.21;**

Male Sprague-Dawley rats were used because of their acceptance as a predictor of toxic change in man. The rats were 6 weeks old at the start of the study.

Estramustine phosphate, in the form of meglumine salt, was administered to groups of rats as a repeated intravenous injection during 3 days. Rats were then sacrificed: a half of the rats at the fourth day and a half at the fifth day.

The dose level of estramustine phosphate, in all the different tested solutions, was of 150 mg/kg/day.

Clinical observations were recorded daily. Thrombophlebitic side effects resulted in a dark bluish/blackish coloration of the tail during the treatment period.

A score system based on tail coloration and its extension was used to evaluate the different tested formulations.

The score system considered estramustine phosphate water solution **(b)** as the positive control (i.e. marked toxicity). Water for injection **(a)** was administered to the control group as negative control (i.e. no toxicity signs).

Histological evaluation was carried out on the tail of the rats treated with the composition of the invention.

Estramustine phosphate in a water solution **(b)** induced, at the used dose, local irritant effects at the injection site after the first administration and marked toxicity signs at the end of the experiment.

Likewise, toxicity signs at the injection site were also observed for the comparison **(c)** and **(d)** EMP solutions containing SBECD or HA, as the sole excipients.

On the contrary, no toxicity was observed with the formulation of the invention containing sulfobutyl ether ß-cyclodextrin and human albumin **(e)**.

Moreover, histological evaluation of the tail of the rats treated with the formulation **(e)** did not reveal any damage when compared to the tails of the control group.

In addition, the synergistic protective effect exerted by combining both SBECD and HA excipients, as per the invention, can clearly be evidenced by considering the formulation of the invention **(e)** in comparison to the formulations **(c)** and **(d)** wherein each single excipient is present in the same amount with respect to the active.

It was thus concluded that estramustine phosphate in a water solution containing sulfoalkyl ether cyclodextrin and human albumin, according to the present invention, induced markedly less local irritant effects when compared with a water solution of estramustine phosphate itself.

Even more surprisingly, the formulation of the invention produced less local irritant effects also in comparison to analogous solutions of estramustine phosphate containing sulfoalkyl ether cyclodextrin only or human albumin only.

One particularly preferred schedule for administering the formulation of estramustine phosphate according to the invention is a single infusion given once weekly to a maximal dose of 4000 mg or 3500 mg/m^2.

Another preferred schedule is the administration of a single drug infusion once every two to four weeks.

One schedule may be preferred over another in consideration of schedules with other optional concomitant therapy. These schedules may repeat in serial or as repetitive fashion.

The formulations of the present invention are useful in antitumor therapy, particularly in the treatment of prostate cancer, breast cancer, melanoma, lung cancer, pancreatic cancer, colorectal cancer, ovarian cancer and cancers of the brain.

The formulations object of the present invention are prepared according to conventional techniques adopted in the preparation of pharmaceutical forms for parenteral use. Typically, a proper amount of estramustine phosphate, either as a dry powder or into a lyophilised form, is dissolved in a pharmaceutically acceptable solution for parenteral use and then admixed with a proper amount of a sulfoalkyl ether cyclodextrin, for instance sulfobutyl ether ß-cyclodextrin.
The above solution is then admixed with a proper amount of human albumin, either as a dry powder or as a commercially available solution, e.g. human albumin 25%, 20% or 5%, optionally properly diluted.
As an example, a proper amount of estramustine phosphate in the form of a suitable salt such as, for instance, N-methyl glucamine salt, is dissolved in a suitable amount of sterile water or aqueous dextrose solution, e.g. 5% dextrose in water for intravenous administration, and then admixed with a proper amount of powdered sulfobutyl ether ß-cyclodextrin.
The above admixture is subsequently added with a proper amount of human albumin, for instance as a dry powder, and subsequently stirred, sterilised and lyophilised according to conventional techniques.
From the foregoing it is clear to the man skilled in the art that each of the ingredients of the invention such as sulfoalkyl ether cyclodextrin and human albumin, each

independently as a powder or into a suitable solution, can be admixed in any order to the active, already dissolved into a proper solution or in the form of a dry powder.
Likewise, the formulations of the invention can also be prepared by admixing the active with the aforementioned ingredients already properly combined as above indicated.
The final freeze-dried formulation is then prepared and stored in vials for injection; the addition of a proper amount of sterile water or a physiological solution for parenteral use enables the preparation of the final formulation to be injected.

The above method is also suitable for preparing high dosages estramustine phosphate formulations whilst maintaining the desired weight ratio between the components.
The unit strength of the formulation to be injected depended on the concentration of the active in the solution itself and, of course, on the filling volume of the vials used to prepare the final formulation.

Additionally, the formulations of the present invention may optionally contain pharmaceutically acceptable excipients for parenteral administration such as, for instance, bulking agents, e.g. lactose or mannitol, pH buffering agents, anti-oxidant agents, preservative agents, tonicity adjusters and the like.

The following examples are herewith intended to better illustrate the present invention without representing any limitation to it.

Example 1

Preparation of estramustine phosphate N-methyl glucamine salt in admixture with sulfobutyl ether ß-cyclodextrin (estramustine phosphate:sulfobutyl ether ß-cyclodextrin=1:1 weight ratio)

300 mg of estramustine phosphate were weighed in a beaker and dispersed by means of magnetic stirring in 5 ml of water. 120.8 mg of N-methyl-glucamine were then added under stirring to the watery dispersion of the active and, after a few minutes, a clear solution was obtained. 312.5 mg of sulfobutyl ether ß-cyclodextrin were added, maintaining the solution under stirring till the solubilization was completed.

The solution obtained was then brought to the final volume of 10 ml with water so as to reach a final concentration of 30 mg/ml of estramustine phosphate and 31.25 mg/ml of sulfobutyl ether ß-cyclodextrin (1:1 weight ratio - 1:0.25 molar ratio respectively).

A solution prepared as previously described, properly sterilized by filtration, was tested for its local vein tolerability in rats.

Example 2

The formulation described in Example 1 was also prepared by solubilization of the commercially available Estracyt® freeze-dried formulation containing 300 mg/vial of the active. The reconstitution of the formulation was made using 10 ml of a 31.25 mg/ml sulfobutyl ether ß-cyclodextrin solution so as to obtain a final concentration of 30 mg/ml of estramustine phosphate and 31.25 mg/ml of cyclodextrin (1:1 weight ratio - 1:0.25 molar ratio respectively).

Example 3

Preparation of estramustine phosphate N-methyl glucamine salt in admixture with human albumin (estramustine phosphate:albumin=1:0.21 weight ratio)

300 mg of estramustine phosphate were weighed in a beaker and dispersed by means of magnetic stirring in 5 ml of water. 120.8 mg of N-methyl-glucamine were then added under stirring to the watery dispersion of the active and, after a few minutes, a clear solution was obtained. 0.250 ml of a

commercially available solution of human albumin at 25% concentration were added whilst maintaining the solution under stirring.

The obtained solution was then brought to the final volume of 10 ml with water so as to reach a final concentration of 30 mg/ml of estramustine phosphate and 6.25 mg/ml of human albumin (1:0.21 weight ratio respectively).

A solution prepared as previously described, properly sterilized by filtration, was tested for its local vein tolerability in rats.

Example 4

The formulation described in Example 3 was also prepared by solubilization of the commercially available Estracyt® freeze-dried formulation containing 300 mg/vial of the active. The reconstitution of the formulation was made by using 10 ml of a 6.25 mg/ml human albumin solution so as to obtain a final concentration of 30 mg/ml of estramustine phosphate and 6.25 mg/ml of human albumin (1:0.21 weight ratio respectively).

The albumin solution could be prepared either by dissolving in water a proper amount of human albumin as a dry powder or by properly diluting a commercially available human albumin solution.

Example 5

Preparation of estramustine phosphate N-methyl glucamine salt in admixture with sulfobutyl ether ß-cyclodextrin and human albumin (estramustine phosphate:sulfobutyl ether ß-cyclodextrin:albumin=1:1:0.21 weight ratio, respectively).

300 mg of estramustine phosphate were weighed in a beaker and dispersed by means of magnetic stirring in 5 ml of water. 120.8 mg of N-methyl-glucamine were then added under stirring to the watery dispersion of the active and, after a few minutes, a clear solution was obtained. 312.5 mg of sulfobutyl ether ß-cyclodextrin were added, maintaining the solution under stirring until complete dissolution.

0.250 ml of a commercially available solution of human albumin at 25% concentration were then added, maintaining the solution under stirring.

The solution was then brought to the final volume of 10 ml with water so as to reach a final concentration of 30 mg/ml of estramustine phosphate, 31.25 mg/ml of sulfobutyl ether ß-cyclodextrin and 6.25 mg/ml of human albumin. The weight ratio between the components of the solution were as follows: estramustine phosphate:sulfobutyl ether ß-cyclodextrin:human albumin 1:1:0.21 respectively.

A solution prepared as previously described, properly sterilized by filtration, was tested for its local vein tolerability in rats.

Example 6

The formulation described in Example 4 was also prepared by solubilization of the commercially available Estracyt® freeze-dried formulation containing 300 mg/vial of the active. The reconstitution of the formulation was made by using 10 ml of a solution containing 31.25 mg/ml of sulfobutyl ether ß-cyclodextrin and 6.25 mg/ml of human albumin so as to reach a final concentration of 30 mg/ml of the active. The weight ratio between the components of the solution were as follows: estramustine phosphate:sulfobutyl ether ß-cyclodextrin:human albumin 1:1:0.21 respectively.